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RHIZOTOXIC EFFECTS OF SILVER IN COWPEA SEEDLINGS

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Abstract—Silver (Ag) is highly toxic to aquatic organisms, including algae, invertebrate animals, and fish, but little information exists on Ag rhizotoxicity in higher plants. In two solution culture experiments with approximately 1,000 μM $Ca(NO_3)_2$ and 5 μM H_3BO_3 (pH 5.4), 20 to 80% of added Ag (≤2 μM) was lost from solution within approximately 30 min, with a further decrease after 48 h root growth. Using measured Ag concentrations at the start of the experiments, the median effective concentration (EC50) for root elongation rate of cowpea (*Vigna unguiculata* [L.] Walp. cv. Caloona) was 0.010 μM Ag in the first 4 h of exposure (0.021 μM in the first 8 h). This demonstrates that Ag (as Ag^+) is rapidly rhizotoxic to cowpea seedlings at concentrations similar to those that are toxic to freshwater biota. Rupturing of rhizodermal and outer cortical layers was evident after 48 h with 0.13 to 0.57 μM Ag initially in solution, being most severe at 0.13 or 0.25 μM Ag. An additional experiment showed that ruptures were first evident after 20 h exposure to 0.17 μM Ag, with increased severity of rupturing over time. The rhizotoxic effects of Ag are similar to those of some other trace metals (e.g., Cu, Al, La) that bind strongly to hard ligands and weakly to soft ligands. The similarity of rupturing effects, despite the difference in strong binding to soft ligands by Ag and to hard ligands by the other metals, suggests a distinctive metabolic effect of Ag that binds only weakly to hard ligands. Environ. Toxicol. Chem. 2010;29:2072–2078. © 2010 SETAC

Keywords—Cowpea Rhizotoxicity Morphology Silver Toxicity

INTRODUCTION

Silver (Ag) is highly toxic to living organisms, and has been classified with mercury (Hg) in the most toxic group of elements [1,2]. Most research has focussed on the toxicity of Ag (specifically Ag^+) to freshwater and marine organisms, such as algae [3], invertebrates [4–6], and fish, including rainbow trout (*Oncorhynchus mykiss* Walbaum) [7,8]. Wood et al. [2] concluded that ''the acute toxicity of Ag (to rainbow trout) appears to be caused solely by ionic Ag^+ interacting at the gills, inhibiting basolateral Na^+, K^+-ATPase activity,'' thereby disrupting osmoregulation. Interestingly, elevated copper (Cu) and aluminum (Al) are also ionoregulatory toxicants [9], but Wood et al. [2] concluded that ''their toxic mechanisms at the gills are different from or are additional to those of Ag.''

The concentration of Ag that is toxic to freshwater biota has been difficult to determine because much information has been presented as total nominal Ag in solution. Lee et al. [3], however, measured soluble Ag in filtered (0.2 μm) solutions, finding a median effective concentration that reduced growth by 50% (EC50) of 0.01 and 0.03 μM Ag for two freshwater algae. Likewise, Bianchini and Wood [5] found a median lethal concentration (LC50) of 0.03 μM Ag for *Daphnia magna* Straus in filtered (0.45 μm), moderately hard water, and Morgan and Wood [8] recorded the same LC50 value for rainbow trout in freshwater (also filtered to 0.45 μm).

Although Ag is strongly bound in soil, Ag toxicity may occur in soils amended with sewage sludge [1], where sediments high in Ag are used for landfill [10], or in sites contaminated with industrial waste high in Ag (e.g., photographic processing). Silver is gaining increased use in medicine and hygiene [11], which may increase its release into the environment. Further studies on the effects of Ag, including the kinetics of symptom development plus their location and appearance, may help elucidate trace metal rhizotoxic phenomena in general. In solution culture, Wallace et al. [12] used much higher concentrations of Ag than those toxic to freshwater biota, reporting that a nominal 100 μM Ag is lethal to bean (*Phaseolus vulgaris* L.) and that 1 μM Ag reduced plant yield by approximately 60%. Leaf necrosis of bean plants grown in solution with 10 μM Ag for 2 d was attributed to a disruption of water transport, but plants partially recovered by 15 d after removal from solution containing Ag [13]. Given that the Ag concentration in solution was not measured in these studies, it is not clear if Ag is as toxic to plant roots as it is to aquatic organisms.

Recent studies have shown that nine trace metals of increasing EC50 for root growth (viz. Cu> indium [In]> Hg = lanthanum [La] = scandium [Sc]> gadolinium [Gd]> ruthenium [Ru] > Al = gallium [Ga]) cause ruptures to the rhizodermis and outer cortex approximately 2 mm behind the root apex (i.e., in the elongation and transition zones) [14,15]. We have also shown using the same technique that the highly rhizotoxic cations, cesium (Cs), nickel (Ni), cadmium (Cd), lead (Pb), and cobalt (Co), which do not bind strongly to hard ligands, do not cause ruptures (unpublished data). These findings suggest that trace metals that bind strongly to hard ligands in the cell wall reduce the ability of the walls of outer cells to loosen and elongate, whereas underlying cells of the stele and inner cortex continue to expand. Indeed, it is noteworthy that a relationship was found between the ability of trace metals to bind to hard ligands, such as the carboxyl groups of pectin within the cell wall, and the formation of ruptures [15]. The comparatively weak binding of Ag to hard ligands [16] suggests

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that it is likely to bind only weakly to the negative charges in root cell walls (which are dominated by carboxyl groups).

Overall, it was hypothesized in the present study that Ag would be highly rhizotoxic, given its toxicity to biota in freshwater systems and its classification in the most toxic group of elements. In keeping with the findings of Kopittke et al. [15], it was also hypothesized that no ruptures would develop because of its low binding strength to hard ligands [16]. Solution culture experiments were conducted to test these hypotheses, to determine the concentration at which Ag is toxic to the roots of cowpea (*Vigna unguiculata*) seedlings, and to study the effects of Ag on root morphology. Results indicate that Ag is indeed highly rhizotoxic and, contrary to expectations, severely ruptured the rhizodermal and outer cortical layers of cells in the root elongation zone. We suggest that these effects are related to the strong binding of Ag to soft ligands, unlike other metals, which rupture roots through reactions with hard ligands.

MATERIALS AND METHODS

Short-term solution culture experiments were conducted using 3-d-old cowpea (*Vigna unguiculata* [L.] Walp.) cv. Caloona seedlings [14]. In experiment 1, seedlings were grown for 16 h in continuously aerated solutions nominally containing only approximately 1,000 μM Ca (as $Ca[NO_3]_2$) and 5 μM B (as H_3BO_3) at pH 5.4. Solution and ambient temperature in the laboratory was maintained constant at 25°C; photosynthetically active radiation was 11 $\mu mol/m^2/s$ at the height of the seedlings for 9 h/d. After this acclimatization period, the seedlings (seven per 650 ml of solution) were transferred to solutions containing the same concentrations of Ca and B plus one of 10 nominal concentrations of Ag set up with aliquots of 0.65 mM Ag as $AgNO_3$ (μM): 0, 0.01, 0.05, 0.10, 0.20, 0.40, 0.60, 0.80, 1.00, or 2.00. These were chosen on the basis of a preliminary experiment that used 10 concentrations of nominally 0 to 150 μM Ag. Solutions were sampled approximately 30 min after addition of Ag stock solution before seedlings were transplanted and at the end of the experimental period 48 h later. The 10-ml samples were filtered to 0.22 μm (Millipore, Millex-GS), acidified with 20 μl concentrated HNO_3 (70%), and stored at 4°C prior to analysis. The concentration of Ca was measured using inductively coupled plasma optical emission spectroscopy (ICP-OES) and that of Ag by inductively coupled plasma mass spectroscopy (ICP-MS) (the latter by two independent laboratories). Unless otherwise indicated, all Ag data mentioned subsequently are those measured by ICP-MS analysis at the beginning of the experimental period. Shortly after seedlings were transferred, the solutions averaged pH 5.4. Seedlings were grown for 48 h, at which time the solutions averaged pH 5.8. Digital images were captured at the time of transfer (0 h) and 4, 8, 12, 24, 36, and 48 h thereafter. The length of each root was measured using UTHSCSA ImageTool 3.0 (University of Texas Health Science Center), available free of charge at http://ddsdx.uthscsa.edu/dig/itdesc.html. These data were used to calculate the root elongation rate (RER) of each root for each time period. All roots were harvested after 48-h growth in Ag solutions, and stored in 10% ethanol in deionized water at 4°C overnight prior to examination using light microscopy without staining. Each treatment had two replicates (a total of 20 beakers), with data being presented as either the mean ± standstandard error (SE) or as nonlinear relationships estimated by GenStat 7.2 [17].

Experiment 2 was a repeat of experiment 1 with the exception that the 2.0 μM nominal Ag treatment was excluded because of its extreme toxicity and a 0.3-μM nominal Ag treatment included to better study milder rhizotoxic effects. Solutions were at pH 5.4 and 5.9 at the start and end of the 48-h experimental period. Data from experiments 1 and 2 were used to estimate the EC50 from the relationship between measured Ag concentration at 0 h and RER after 4 and 8 h growth.

Experiment 3 used the procedures of the previous experiments to examine the effects of a single Ag concentration on root growth and morphology. After an initial acclimatization period of 16 h, seedlings (seven per 650 ml of solution) were transferred to solutions with nominally 0.4 μM Ag. Solutions were sampled before transferring the seedlings (0 h) and 48 h later, followed by filtering (0.22 μm) and acidification. Mean solution pH was 5.3 at the beginning of the experiment, increasing to a mean of pH 5.6 over the eight harvest times (4, 8, 12, 16, 20, 24, 36, and 48 h). After capturing digital images, three roots were randomly sampled for light microscopy (as in experiments 1 and 2) and the remaining four for scanning electron microscopy (SEM) [14]. These root tips (~15 mm) were pinned to a Styrofoam block, placed in liquid nitrogen for 10 s, and immediately transferred to approximately 30 ml 3% glutaraldehyde in methanol solution at −20°C for 24 h. After immersion in methanol (without glutaraldehyde) at −20°C for a further 24 h, the roots were warmed to room temperature, dried using a Balzers critical point drier, and coated with approximately 15 nm of platimun (Pt) for examination with a field emission SEM (JEOL JSM 6400F) at 10 kV and 39 mm working distance.

Experimental data from the present study on Ag were compared with those using the same technique that evaluated the rhizotoxic effects of Al, Cu, and La [14] and of Ga, Gd, In, Hg, Ru, and Sc [15]. The rhizotoxic effects of these and other metals (unpublished data) were compared using binding strengths determined by Kinraide [16]. The hard ligand scale (HLScale) is based upon the strength of the ion's binding to 13 hard ligands (e.g., oxalate, sulfate, and fluoride). The soft ligand scale (SLScale) is based upon the strength of the ion's binding to seven soft ligands such as sulfide, thiourea, and iodide. These scales are normalized using the mean and standard deviation (SD) (0 = the mean, 1 = one SD above the mean, and −1 = one SD below the mean) and are proportional to $\log_{10}$ of published binding constants [16]. Insufficient data are available (see Table 2 of Kinraide [16]) for inclusion of Ru^{3+} in either the HL or SLScale; likewise for five other ions (Al^{3+}, Gd^{3+}, La^{3+}, lithium [Li^+], and Sc^{3+}) in the SLScale. In these cases, values were computed from regression equations incorporating physical parameters of the ions (e.g., charge and Pauling electronegativity) [16]; r^2 values were 0.866 to 0.903.

RESULTS AND DISCUSSION

The measured concentration of Ca in solution was as expected, 990 ± 30 μM in experiment 1 and 980 ± 60 μM in experiment 2 (mean ± SE of values at the start and end of each experiment). However, measured Ag in solution was substantially lower than the nominal concentration at the start of both experiments (Fig. 1a). In experiment 1, measured Ag in solution was generally 20 to 50% of the nominal Ag concentration; corresponding values were slightly higher in experiment 2, 40 to 80%. The measured concentration of Ag in solution was even lower by the end of the experimental period (Fig. 1b), with <10 to 20% of the nominal Ag present in solution at the end of experiment 1 and from 10 to 35% in experiment 2. Measured concentrations were 1.3 and 0.48 μM Ag at 0 and 48 h in the

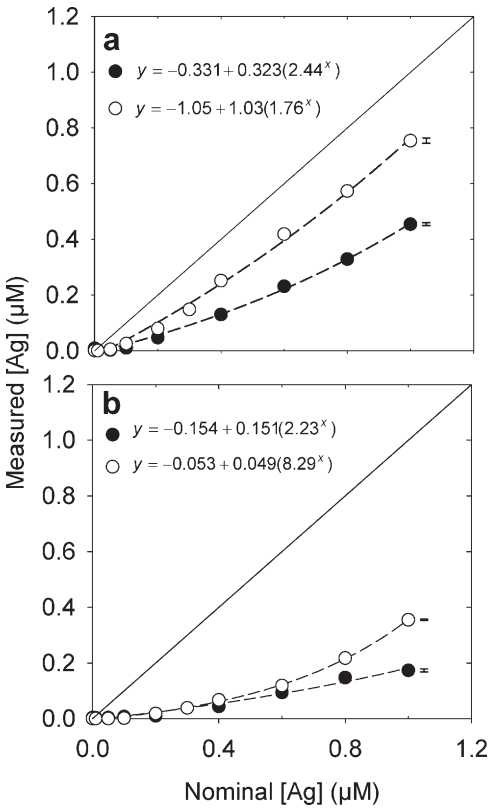

Fig. 1. Relationships between nominal and measured concentrations of Ag at the start (0 h) (**a**) and end (48 h) (**b**) of the experimental period in experiments 1 (●) and 2 (○). The fitted curves (------) are compared to the solid 1:1 line. The vertical bars represent the standard errors of the fitted curves based on two replications. Each fitted curve provided a highly significant R^2 value ≥0.976.

highest Ag treatment in experiment 1. Exponential relationships between nominal and measured values were fitted to describe Ag present in solution (Fig. 1). The shape of these curves suggests that loss of Ag from solution was due to Ag sorption by the beaker at the start of the experiment and possibly by roots also by the end of the experiment. This agrees with reported losses after 44 h of approximately 50% of 0.46 μM Ag originally in solution in borosilicate glass flasks and even greater percentage loss at lower Ag concentrations after 18 h incubation in borosilicate glass cavity slides [18]. Similarly, Bianchini and Wood [5] found that only 47 ± 9% of the original 0.01 to 0.21 μM Ag remained in moderately hard water with composition (mM) of 0.8 sodium (Na), 2.2 K, 1.0 calcium (Ca), 0.1 magnesium (Mg), and 0.6 chlorine (Cl).

Such findings stress the importance of measuring the concentrations of trace metals in solution. Indeed, Lee et al. [3] concluded from a literature search of Ag effects in aquatic systems that the ''compilation is of limited value since the EC50 values were all reported as nominal total silver concentrations, with no consideration of silver speciation or of silver loss from the exposure media during the toxicity tests.'' In the present study, GEOCHEM-EZ (available on the internet at http://www.PlantMineralNutrition.net) [19] indicated that 100% of soluble Ag was present as Ag^+ with an activity coefficient of 0.94. This enables conversion of measured Ag concentration used here to Ag^+ activity.

The detrimental effect of Ag to cowpea root growth was visible after 12-h exposure to the higher levels of Ag. After 24 h, the tips of roots growing in solutions with 0.13 and 0.25 μM Ag measured in experiments 1 and 2 were visibly deformed, with ruptures in the region behind the apex. These roots and those at higher Ag concentration were light brown. In the preliminary experiment, roots in solution with nominally ≥5 μM Ag did not elongate, and removal from solution showed that they were flaccid.

Root length measurement in experiments 1 and 2 showed good growth in the control solution, RER ranging from 1.2 to 1.5 mm/h over the 48-h experimental period. Except for the 0.003 μM Ag treatment, increasing Ag in solution was markedly detrimental in the first 4 or 8 h of exposure (Fig. 2). Using data from these initial 4- or 8-h periods, an EC50 was estimated as 0.010 or 0.021 μM Ag in solution. This indicates that Ag is as toxic to root elongation of cowpea as it is to freshwater biota and is more toxic than Hg (EC50 = 0.59 μM), the most rhizotoxic of nine other metals in cowpea tested under similar conditions [14,15]. Complete cessation of root growth was not evident within 4 h even at the highest concentrations of 1.3 and 0.75 μM Ag in solution (Fig. 2a), but this was probably due to some root growth soon after transfer. This was evident by evaluating the effect of Ag on RER over the first 8 h of exposure (Fig. 2b).

Recovery of roots from the toxic effects of Ag at low concentrations (0.004 to 0.015 μM) was almost complete as the experiments progressed (Fig. 3). This was most likely due to the loss of Ag from solution between the start to the end of the experimental period (Fig. 1). Wallace and Mueller [13] had shown that bean roots recover after removal from solutions containing Ag. Roots continued to grow, albeit slowly, at 0.42 ± 0.10 mm/h with 0.047 μM Ag decreasing further to 0.12 ± 0.11 mm/h with 0.13 μM Ag. Above this concentration, an almost complete cessation of root growth occurred within 12 h of exposure.

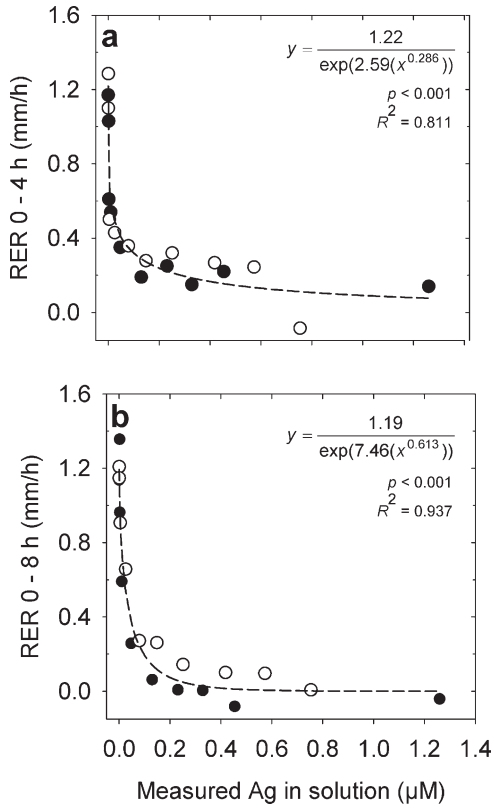

Fig. 2. Effect of measured Ag in solution at the start of experiments 1 (●) and 2 (○) on the root elongation rate (RER) of 3-d-old cowpea seedlings during the first 4 h (**a**) and 8 h (**b**) of exposure, with the fitted line (------) estimating the decrease in RER with increase in measured Ag in solution.

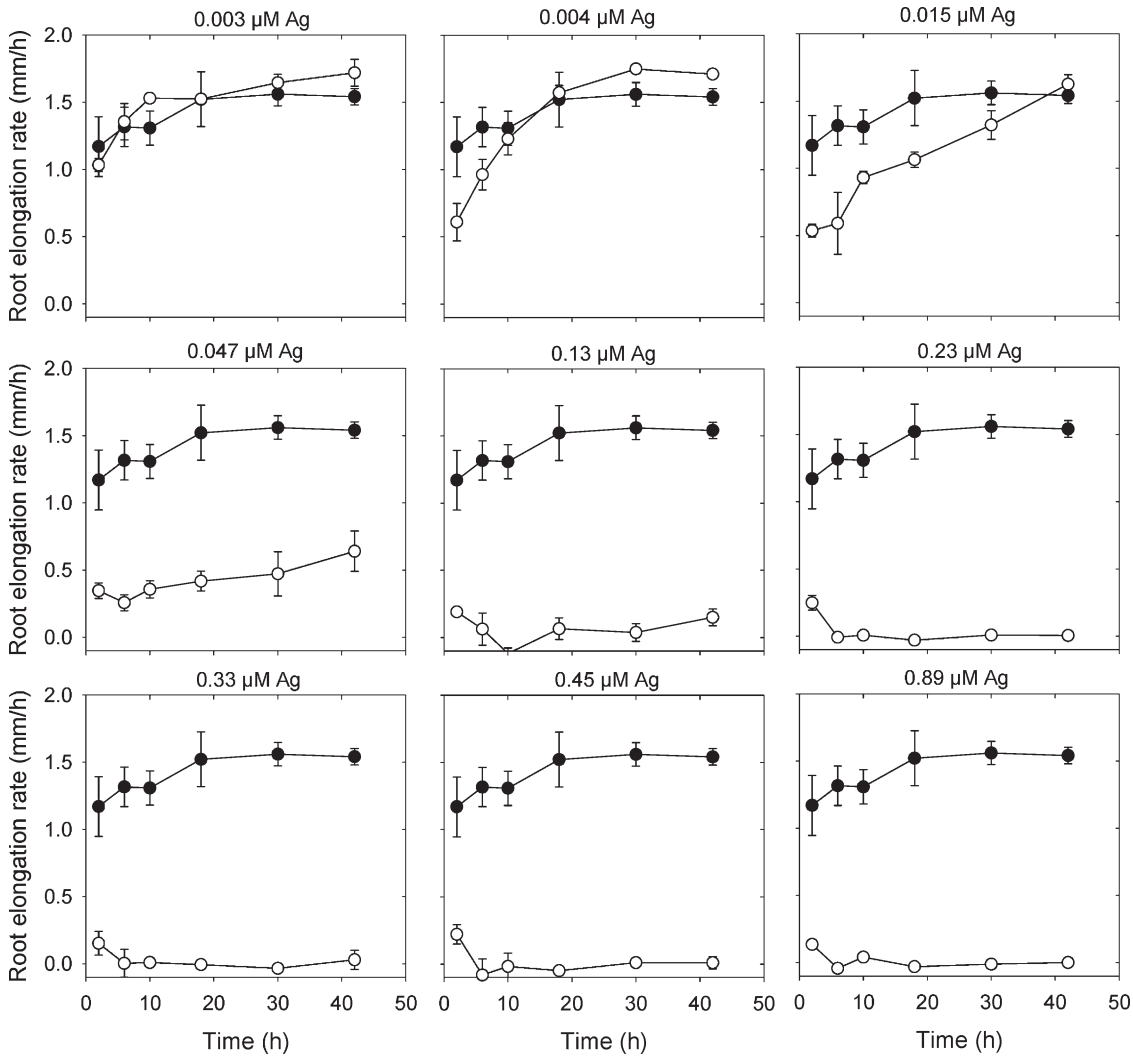

Fig. 3. Root elongation rate of 3-d-old cowpea seedlings in solutions containing approximately 1,000 µM $Ca(NO_3)_2$, ca. 5 µM H_3BO_3 and measured concentrations of Ag (○) at the start of the experimental period (0 h) (experiment 1) compared with the control treatment (●) with no added Ag. The vertical bars represent the standard errors of the means of two replicates; where not visible, the standard error is less than the size of the symbol.

Light microscopy revealed healthy root growth in the absence of added Ag (Fig. 4a) and no abnormalities of roots grown with <0.02 µM Ag in solution. With measured concentrations in experiment 1 of 0.02 and 0.04 µM Ag, however, the surface of the roots appeared scruffy (Fig. 4b and c). Peeling of epidermal cells was evident in some roots suggesting damage to this tissue, although some adherence of root cap cells and mucilage to the outside of the root may have occurred. All roots grown for 48 h at 0.13 µM Ag (experiment 1) were severely ruptured over a length of approximately 4 mm starting approximately 0.5 mm from the apex (Fig. 4d). Only a single rupture approximately 2 mm from the apex occurred in 60% of roots grown at 0.25 µM Ag or 10% of roots grown at 0.38 µM Ag (Fig. 4e and f). The results of experiment 2 confirmed those of experiment 1 (data not shown). In the solution containing 0.15 µM Ag, roots were either scruffy (as in Fig. 4b and c) or ruptured (as in Fig. 4d). Severe rupturing occurred at 0.25 µM Ag, the measured concentration at which only a single rupture was evident in experiment 1 (Fig. 4e). The brown discoloration of roots was clearly evident in the light micrographs at 0.13 to 0.53 µM Ag (Fig. 4d–g) but, surprisingly, no discoloration and no rupturing of roots in solutions at the highest concentration of 1.3 µM Ag in experiment 1 was found despite cessation of growth (Fig. 4h).

In experiment 3, the mean Ca concentration was $1{,}000 \pm 50$ µM and that of Ag at the start of the experiment was 0.17 ± 0.01 µM; 0.08 ± 0.03 µM over the eight harvest times. A RER of 1.37 ± 0.11 mm/h was determined without added Ag; this was only 0.11 ± 0.06 mm/h at 0.17 µM Ag (i.e., a 92% decrease in RER). Previous studies [14,15] had shown that trace metals differ in their effects on the kinetics of rupture initiation, ranging from 2 h after the start of exposure to Al, In, Ru, or Sc up to 24 h with Hg. The results of experiment 3 suggest that Ag behaves like Hg in this respect, with ruptures first evident with light microscopy only after 20 h in solutions with Ag (data not shown). This was confirmed by higher resolution SEM. The first ruptures evident after 20 h were shallow, few in number, and located approximately 1 mm from the apex (Fig. 5a and d). Some roots were swollen and kinked, with ruptures evident on the convex side of the kink; in others, the tips were markedly thinner than older parts. Over time, the severity of rupturing increased in that more ruptures appeared over a longer distance (Fig 5a, b, and c), they were deeper (up to 0.2 mm) (Fig. 5e), and some were located closer to the apex (Figs. 4 and 5c). A longitudinal rupture was evident in

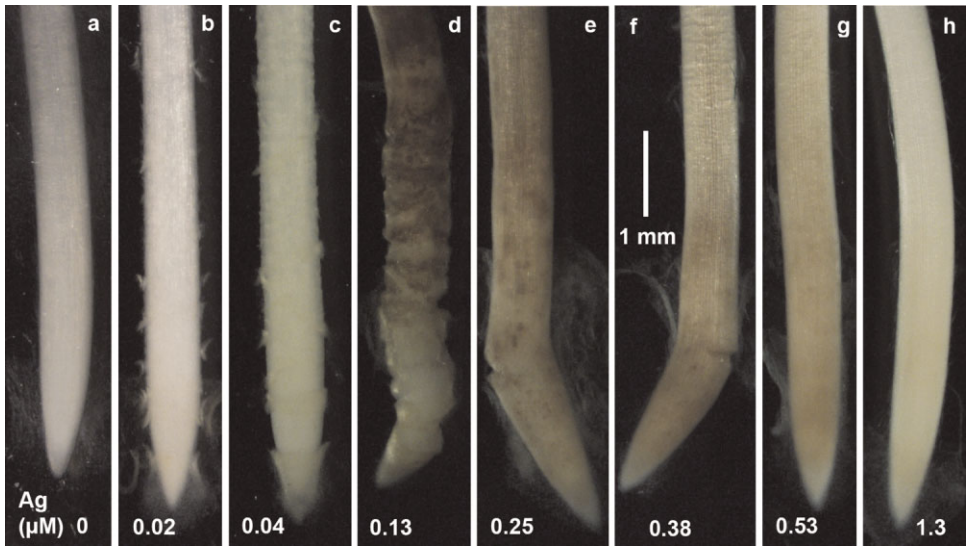

Fig. 4. Light micrographs of unstained cowpea root tips after 48 h growth in solutions containing ca. 1,000 μM $Ca(NO_3)_2$, ca. 5 μM H_3BO_3 and measured concentrations of Ag at the start of the experimental period in experiment 1. The 1-mm scale bar applies to all micrographs. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com.]

the rhizodermis of some roots (Fig. 5a and d) but was not observed using light microscopy.

Rupturing in the root elongation zone appears to coincide with continued root growth, although at a low RER (0.11 ± 0.06 mm/h at 0.17 μM Ag in experiment 3, for example) and over a narrow range in Ag concentration. The explanation [14,15] seems valid, therefore, that ruptures develop in the rhizodermis and outer cortex of the elongation zone because the walls of the outer cells do not relax in a controlled manner [20], whereas the cells of the stele and inner cortex continue to elongate. Nevertheless, the observation that Ag causes ruptures was unexpected, given that the ability of trace metals to cause ruptures was considered to be related to the strength with which they bind to hard ligands, likely the carboxyl groups of pectin [15]. Rather, Ag binds strongly to soft ligands (SLScale of 1.13) but only weakly to hard ligands (HLScale of −1.28) [16]. Thus, the observation that Ag causes similar symptoms to Al, Cu, Ga, Gd, Hg, In, La, Ru, and Sc is intriguing, given that Ag presumably binds to different ligands within the root compared to these cations except that Hg binds strongly to both hard and soft ligands. This suggests that Ag is unique in binding strongly to soft ligands but has a similar effect (i.e., rupturing) to that of other metals that bind strongly to hard ligands, possibly by disrupting the same physiological process. Several enzymes in the apoplast function in the synthesis and rearrangement of cell wall materials during cell elongation [21]. One or more of these enzymes may be vulnerable to elevated concentrations of metal ions able to bind strongly to either hard-ligand (e.g., carboxyl

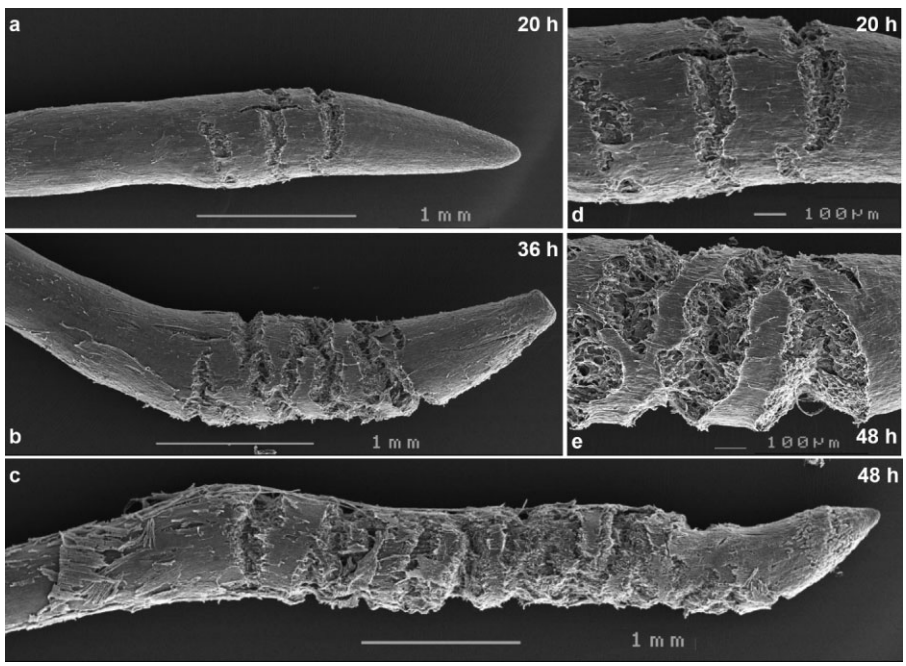

Fig. 5. Scanning electron micrographs of cowpea seedling root tips grown for 20 to 48 h in solutions containing ca. 1,000 μM $Ca(NO_3)_2$, ca. 5 μM H_3BO_3, and 0.17 μM Ag as $AgNO_3$ illustrating the increasing severity of rupturing over time (**a, b, c**) and, at higher magnification, the increased number and depth of ruptures in the elongation zone (**d, e**) from 20 to 48 h (experiment 3).

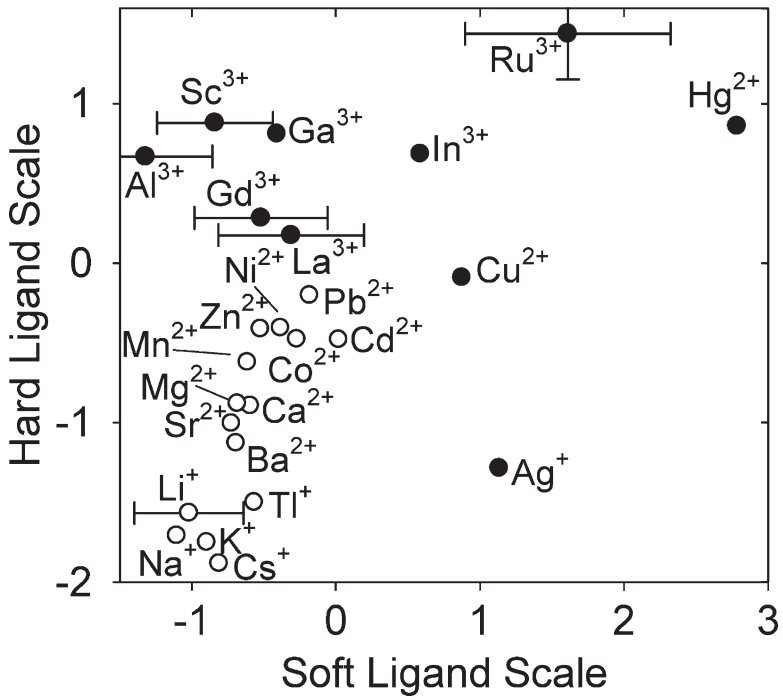

Fig. 6. Classification of cations according to strength of binding to soft and hard ligands tested in short-term experiments with cowpea cv. Caloona seedlings using the same procedure [14,15], with solid circles (●) indicating cations that cause ruptures to the rhizodermis and outer cortex and open circles (○) cations that do not. The 95% confidence intervals are included for the computed Soft and Hard Ligand Scale values for Ru^{3+} and the Soft Ligand Scale value for Al^{3+}, Gd^{3+}, La^{3+}, Li^{+}, and Sc^{3+}; all other values were measured [16].

groups) or soft-ligand moieties in the enzyme (e.g., thiol groups). Interestingly, it appears that cations that do not bind strongly to either soft or hard ligands (i.e., cations in the bottom-left quadrant of Fig. 6) do not cause ruptures.

Hasenstein and Evans [22], Kollmeier et al. [23], and Hong et al. [24] have suggested that auxin plays a role in the expression of Al toxicity. An inhibition of basipetal auxin transport in the rhizodermis and outer cortex ([25]; http://www.aspb.org/publications/arabidopsis/; [26]) may reduce the ability of these cell walls to loosen as required according to the acid growth hypothesis [26]. This, coupled with continued elongation of the underlying cells, would result in ruptures. Thus, we propose several interrelated hypotheses relating to auxin transport to explain the similarity in symptoms caused by Ag and by Al, Cu, Ga, Gd, Hg, In, La, Ru, and Sc. For trace metal cations that bind strongly to hard ligands (including carboxyl, hydroxyl, phosphoryl, sulfate, and amine groups), binding to the carboxyl groups of pectin may reduce loosening of the cell wall directly through increased crosslinking of the cell wall polymers [27,28]. Alternatively, increased crosslinking of pectin by strongly bound cations or high concentrations of cations minimizes enzymatic attack on the pectin backbone [29]. Indirect action may occur through a reduction in pore size (with a consequent decrease in apoplastic flow) or by reducing the effects of cell wall acidification via exchange for Ca^{2+} [30]. It is also possible that these cations bind directly to the auxin molecule itself (the negative charge of indole acetic acid [IAA] results from dissociation of a carboxyl group [26]), a reaction that would limit the counter transport of the IAA^{-} ion.

Cations such as Ag bind strongly to soft ligands, including sulfhydryl groups, olefins, or aromatic groups, and may bind to these groups in the cell wall [31]. It is possible also that the binding of Ag either inhibits the transport of IAA^{-} across the plasmalemma or interferes with H^{+}-ATPase in a manner similar to the inhibition of basolateral Na^{+}, K^{+}-ATPase activity (which disrupts osmoregulation) in the gills of rainbow trout [2]. Both these scenarios would prevent acidification of the apoplast. A final possibility involves Ag binding strongly to proteins with soft-ligand moieties (endoglucanases, expansins) involved in loosening of the cell wall, rather than to the substrate as occurs with metals that bind to strong ligands of pectin [29].

CONCLUSIONS

The highly toxic effect of Ag on cowpea root growth (EC50 = 0.010 to 0.021 μM Ag in the initial 4 or 8 h) was expected, given reports of Ag toxicity in freshwater aquatic systems [1,2], with Ag rhizotoxic at concentrations similar to those that are toxic to freshwater biota. In contrast, the ruptures that developed on exposure of roots to Ag were not expected in view of the low binding strength of Ag to hard ligands [16]. This makes Ag unique among the metals tested under similar experimental conditions. Ruptures to the rhizodermis and outer cortex developed in a narrow range of Ag in solution of 0.13 to 0.53 μM Ag (experiment 1) and 0.25 to 0.57 μM Ag (experiment 2). Reasons for rupturing to the rhizodermis and outer cortex may be connected with strong binding of Ag to the cell wall [31], inhibition of enzymes required for cell expansion, or a disruption of basipetal auxin transport in the outer cell layers via a number of mechanisms.

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